What's in That Package? An Evaluation of Quality of Package Honey Bee (Hymenoptera: Apidae) Shipments in the United States

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J. Econ. Entomol. 101(3): 668–673 (2008)

ABSTRACT To replace deceased colonies or to increase the colony numbers, beekeepers often purchase honey bees, *Apis mellifera* L., in a package, which is composed of 909–1,364 g (2–3 lb) of worker bees and a mated queen. Packages are typically produced in warm regions of the United States in spring and shipped throughout the United States to replace colonies that perished during winter. Although the package bee industry is effective in replacing colonies lost in winter, packages also can be an effective means of dispersing diseases, parasites, and undesirable stock to beekeepers throughout the United States. To evaluate the quality of packages, we examined 48 packages representing six lines of bees purchased in the spring 2006. We estimated levels of the parasitic mite *Varroa destructor* Anderson & Trueman and the percentage of drone (male) honey bees received in packages. We surveyed for presence of the tracheal honey bee mite, *Acarapis woodi* (Rennie), and a microsporidian parasite, *Nosema* spp., in the shipped bees. We found significant differences in both the mean Varroa mite per bee ratios (0.004–0.054) and the average percentage of drones (0.04–5.1%) in packages from different producers. We found significant differences in the number of *Nosema*-infected packages (0.0–75.0%) among the six lines. No packages contained detectable levels of *A. woodi*. Considering the observed variability among honey bee packages, beekeepers should be aware of the potential for pest and disease infestations and high drone levels in packages.

KEY WORDS *Apis mellifera*, *Varroa destructor*, *Acarapis woodi*, *Nosema* spp., package honey bees

The honey bee, *Apis mellifera* L., is an economically important arthropod due to the value of hive products and pollination service that this insect provides (Morse and Calderone 2000). Although colonies are typically maintained perennially, apiculturists often need to restore colony numbers that have been depleted by winter mortality. To service this need and to supply beekeepers who wish to increase the number of colonies they manage, an industry has developed that supplies "package bees" that beekeepers can use to start new colonies. A package of bees is typically sold as 909 g (2 lb) or 1,364 g (3 lb) of worker bees and a young unrelated mated queen raised by the producer (Laidlaw 1992). The package bee industry is largely based in the southeastern United States and California where warm spring climates allow producers to raise a surplus of bees and provide mated queens that can be shipped throughout the United States in spring. More recently, packages of bees have been shipped from Australia to the United States to provide supplemental colonies for early season pollination of California almond (*Prunus* spp.) trees (Harrison 2005).

The exact methods used to make packages vary among producers, but the basic practices are detailed by Laidlaw (1992) who described the industry standard for production of packaged bees. Briefly, populous honey bee colonies are disassembled and worker bees are shaken from the hive into a large mesh cage creating a mixture of workers from many colonies. Two or three pounds of bees are then transferred from the large cage to a smaller "package," a wooden box with two screened sides. A smaller queen cage, containing a young mated queen, is suspended inside the package. The confined bees are provisioned with up to a liter of sugar syrup for shipment, which is sufficient to sustain the bees for ≈1 wk. Workers feed the queen during transport. Upon receipt by the beekeeper, package bees are installed in a hive (Delaplane 1994), and they subsequently establish a colony that is headed by the young queen shipped with the bees.

Although package bees are a simple and effective way to transport bees over a long distance, the shipment of bees is not without problems and risks. Even from reputable producers, 4% queen mortality was routinely observed during shipments of 50–150 packages of bees over a 3-yr period (J.P.S., unpublished data). In some cases, whole packages perish when left in the sun or otherwise overheated during shipment. Rough handling by shippers can cause the queen cage to become free from the package cage and roll around

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in the package, resulting in queen mortality (unpublished data).

In addition to direct damage to the bees during shipping, parasites and diseases may be shipped with the package to the beekeeper (Wehling 2000, Hayes 2006). The ectoparasitic mite *Varroa destructor* Anderson & Trueman is presently the most economically important pest of *A. mellifera.* The adult mites are phoretic on adult bees and are easily transported when bees are moved. Infested honey bee colonies, left untreated, develop levels of mites that cause reduction in adult bee population, reduced brood production and ultimately, colony death (De Jong et al. 1982a, Delaplane and Hood 1997, Strange and Sheppard 2001). Ideally, the level of *V. destructor* in package bees should be well below the local treatment threshold where the package is to be installed; if the new colony meets or exceeds the threshold, the beekeeper will need to apply a mite control treatment immediately after the package is installed. Because treatment thresholds vary regionally (Delaplane and Hood 1997, Strange and Sheppard 2001), mite levels at the package destination may exceed the local treatment threshold even though the mite levels at the production location were below the treatment threshold.

In addition to *V. destructor,* the tracheal honey bee mite, *Acarapis woodi* (Rennie) (Liu and Nasr 1993); the small hive beetle, *Aethina tumida* Murray (Baxter et al. 1999); and the protozoan parasites (*Nosema apis* Zander or *Nosema ceranae* Fries et al.) may be transported along with the host. Sundry other parasites and diseases (including viruses) may be transported along with the bees, leading to novel introductions or reinfestation of a beekeeper's stock. The danger of pest and pathogen movement is underscored by recent colony declines (due to so-called colony collapse disorder), which may be linked to Israeli acute paralysis virus (family *Dicistroviridae,* genus *Iflavirus,* IAPV) thought to be imported in Australian-derived honey bee packages (Cox-Foster et al. 2007), although the exact origin of IAPV in North America is unknown.

Despite the fact that a package is primarily a way to establish a new colony consisting of a queen and worker bees, some drone (male) bees are likely to be included. Producers can exclude most drones from packages by shaking the bees through a queen excluder grill through which drones are too large to pass. Because drones contribute little to the productivity of a colony, the inclusion of large numbers of drones in a package of bees may reduce the overall productivity of the new colony. Drones effectively reduce the size of the worker force in a package and place a greater strain on the resources of the developing colony as they must be fed from the available pool of resources, but they contribute nothing to resource collection. Producers that do not use a queen excluder when making up packages will have a proportion of drones in packages similar to the proportion of drones in the mature colonies from which they are constructed.

Along with the quality of the worker bees in a package, the quality of the new queen is important to the success of the new colony. Queen honey bees sold with package bees are often marketed as being of pure race (e.g., Italian, Carniolan) or as selected hybrids (e.g., Buckfast, Russian, VSH [formerly SMR]). Queens of pure race or hybrid origin are usually selected from genetic lines maintained by the producer. Although the worker bees in a package are likely unrelated to the queen with whom they are shipped, it is ultimately that queen's offspring that will constitute the colony (Laidlaw 1992); and so, packages are marketed as coming from a particular genetic stock. The change from package workers to the queen's offspring takes ≈6 wk to complete because the new queen's first brood does not emerge for >21 d after installation into the hive and old package workers live for ≈6 wk (Delaplane 1994).

The current study assesses the quality of 48 honey bee packages in which we estimated levels of *V. destructor, A. woodi,* and *N. apis* (or *N. ceranae*) shipped with the bees and the percentage of drones shipped in the packages. We evaluate the quality of queens shipped in the packages 6 wk after installation, noting queen mortality or supersedure by a new queen.

Materials and Methods

We received eight 1,364-g (3-lb) packages from each of six different genetic stocks or lines of honey bees (hereafter referred to as lines 1–6) purchased from four package bee producers (named producers A–D) in the southern United States in May 2006. Packages were initially ordered from two additional sources; however, those packages were not shipped. Producers were not informed of the inclusion of their product in the current study, and orders were placed only with producers who would ship directly to our location. Packages were installed on single-story, 10-comb, full-depth (≈24.45-cm) Langstroth equipment in Ithaca, NY, within 48 h of arrival at the local post office. A sample of 192–570 (mean 316) bees was collected from each package at the time of installation and stored in a 250-ml bottle with 95% ethanol. The samples were later used to estimate the *V. destructor* mite-per-bee ratios (MPB), *A. woodi* parasitism, *Nosema* infection, and percentage of drones in the packages. New ethanol was added after MPB ratios were determined to replace ethanol lost during processing. Samples were labeled and stored at room temperature except when bees or mites were removed for analyses.

Queens shipped with the packages were marked on the thorax with a dot of colored enamel upon introduction to the colony so they could be easily located during subsequent inspections. The colonies were inspected ≈3 and 6 wk after package installation for colony condition and the presence of the original marked queen.

***V. destructor* Assessment.** Varroa mites were separated from the bees by using the alcohol wash method (De Jong et al. 1982b). For each sample, a wire-mesh screen was installed in the neck of the 250-ml sample bottle, and the bottle along with bees and alcohol were affixed to a wrist action shaker and agitated for 60 min. Bees were retained above the screen, whereas mites fell through the screen into the bottle cap. Bees and mites were counted, and the bees were visually inspected during counting to ensure thorough removal of mites. The number of mites was divided by the number of bees to give the MPB. Because beekeepers frequently use a field assay called an ether roll to assess mite levels, MPB ratios were converted to a standard 300 bee ether roll (SER) count with the formula SER = $[(R \times B)/1.783]/(B/300)$, where R is the MPB ratio, B is the number of bees in the sample, and 1.783 is the conversion factor (Caldron and Turcotte 1998).

Tracheal Mite Assessment. Tracheal mite infestation rates were determined for each package by microscopic visual inspection of dissected prothoracic tracheae (Calderone and Shimanuki 1992, 1993) of 25 workers from each sample. The number of mites in each individual honey bee was recorded.

***Nosema* Infection Assessment.** *Nosema* infections were detected by polymerase chain reaction (PCR) amplification of homogenized gut tissue removed from 15 workers from each package (Shimanuki and Knox 2000). Digestive tract tissue was dissected from the 15 workers, frozen in liquid nitrogen, and homogenized in 1.5-ml microcentrifuge tubes by using a plastic pestle. The resulting homogenate was resuspended in 100 μl of water, vortexed, centrifuged (16,000 $\times$ *g* for 10 min), and the supernatant removed. A Chelex extraction protocol was used to extract DNA (Walsh et al. 1991, Klee et al. 2007). DNA was amplified using the SSUrRNA primers and protocols described by Klee et al. (2007), and bands were visualized on a 1.4% agarose gel. Samples that displayed a band were scores as "infected," and samples lacking a band were scored as "disease-free." All samples were run alongside a positive control of honey bee gut with a known infection.

Proportion of Drones in Packages. The bees in each sample were scored as either worker or drone as they were counted. The proportion of drones in the sample was calculated by dividing the number of drones in the sample by the total number of workers plus drones in the sample.

Statistical Analysis. Proportion data (MPB ratio and proportion of drones) were transformed using the arsine $\sqrt{Y}$ to meet the assumptions required for the analysis. A univariate analysis of variance (ANOVA) (SPSS Inc. 2006) was used to test for differences in 1) MPB ratios and 2) proportion drones, with line as the fixed factor. Means were compared with pair wise post hoc least significant difference (LSD) tests, and significance was set at the $P < 0.05$ level for all comparisons. Differences in queen mortality among lines and in the number of *Nosema*-infected packages per line were compared with a nonparametric Kruskal–Wallis test, with significance set at the $P < 0.05$ level.

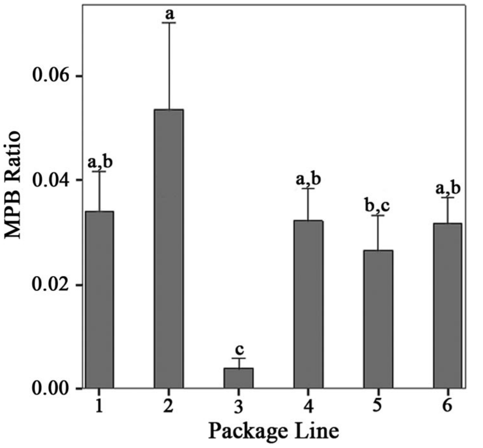

Fig. 1. Mean MPB ± SEM for each of the six tested lines of package bees. For each bar $n = 8$ packages. Means represented by bars with different letters were significantly different ($P < 0.05$) based on LSD comparison.

Results

Queen Survival. There was no significant difference in queen survival rates (Kruskal–Wallis rank test; $\chi^2 = 2.136$, df = 5, $P = 0.83$) 6 wk after package installation. Four of the six lines in the experiment each lost a single queen (87.5% survival), whereas two lines had 100% queen survival. One queen was superseded by a new queen; two queens died within a week of installation with no queen replacement; and one queen began laying unfertilized drone eggs, resulting in the collapse of that colony.

***V. destructor* and *A. woodi*.** MPB ratios (Fig. 1) were significantly different among the six package lines ($F = 5.65$, df = 5, $P < 0.001$). The range among lines (mean ± SEM) (Table 1) was from 0.004 ± 0.005 to 0.054 ± 0.047 MPB. These ratios represent approximately four mites per 1,000 bees in the line with the lowest mean MPB ratio to 54 mites per 1,000 bees in the line with the highest mean MPB ratio. No varroa mites were detected in three packages (all from one producer); the highest ratio observed among all packages was 0.134 MPB. The mean MPB ratio of all 48 packages was 0.030 ± 0.027. Line 3 had significantly fewer mites than the four other lines, and line 2 had

Table 1. Rate of queen failure, standardized 300 bee ether roll count (mean ± SEM) and percentage of *Nosema*-infected packages in different lines of honey bees from four producers of package honey bees

Producer	Line (no. packages)	Queen failure (%)	Varroa SER	No. *Nosema*-infected packages
A	1 ($n = 8$)	12.5	5.71 ± 1.30	2
B	2 ($n = 8$)	12.5	9.01 ± 2.81	3
C	3 ($n = 8$)	0.0	0.67 ± 0.30	0
D	4 ($n = 8$)	12.5	5.42 ± 1.05	0
D	5 ($n = 8$)	12.5	4.46 ± 1.13	6
D	6 ($n = 8$)	0.0	5.35 ± 0.84	4
	All ($n = 48$)	8.33	5.10 ± 0.67	15

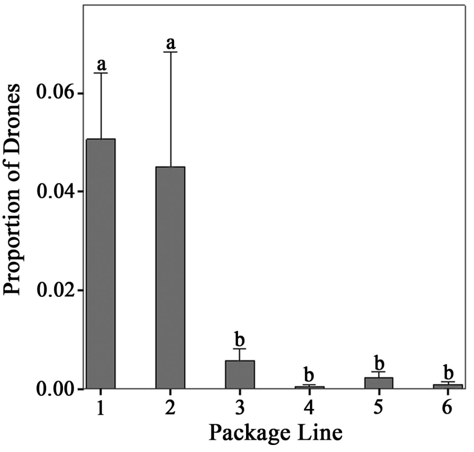

Fig. 2. Mean proportion of drones ± SEM in samples from each of the six tested package lines. For each bar n = 8 packages. Means represented by bars with different letters were significantly different ($P < 0.05$) based on LSD comparison.

significantly more mites than lines 3 and 5. MPB ratios converted to SER values (Calderone and Turcotte 1998) are reported in Table 1. No tracheal mites were found in any bees sampled.

***Nosema* Infection.** There were significant differences in the number of *Nosema*-infected packages among the six lines (Kruskal–Wallis rank test; χ^2 = 15.67, df = 5, P = 0.008). Samples from 15 of the 48 (31.25%) packages we tested produced bands indicating *Nosema* infection (Table 1). Within lines 3 and 4, we detected no infected packages, whereas the other lines had two, three, six, and four samples with infected bees, respectively. Although we did not directly quantify the severity of the infection in individual bees or the percentage of infected individuals per package; post-PCR microscopic inspections of gut samples and the florescent intensity of the amplified band of DNA indicated that the severity of infection was variable among packages.

Proportion of Drones in Packages. Lines 1 and 2 contained significantly larger proportions of drones (Fig. 2) than the other four lines (F = 11.166, df = 5, $P < 0.001$). In addition to having higher numbers of drones, we observed a high degree of variability in lines 1 and 2 with one package composed of 20.3% drones (Table 1). Drone levels in the other four lines with significantly fewer drones ranged from 0 drones (20 packages) to 1.9% drones, and they were not significantly different from one another.

Discussion

We found package quality (as measured by MPB, percentage of drones, and *Nosema* prevalence) to be highly variable both among and within the lines we surveyed. Of the six lines of bees for which we received packages, line 3 had no queen mortality in the first 6 wk, the lowest MPB scores, no detected *Nosema*, and the lowest percentage of drones. Line 4 had low drone levels, intermediate MPB levels, and no *Nosema;* yet, lines 5 and 6 also from producer D had intermediate MPB ratios and low drone numbers, but >50% of those packages had detectable *Nosema* infections. Lines 1 and 2 had high MPB ratios, high drone numbers and >25% of the packages in each line had *Nosema* infections. The current study did not attempt to qualify the resulting colonies (excepting short term queen survival) derived from the package shipments, but only the variability within the packages we received. Additionally, our sample of packages was limited to four producers so some question remains as to whether the current study included the extremes in package quality or is generally representative of the packages available to beekeepers. Nevertheless, we observed that some producers are shipping packages with higher MPB ratios, higher proportions of drones, and a higher incidence of *Nosema* infection than other producers.

The high variances associated with both MPB ratios and the proportion of drones among producers is likely indicative of the variation in production practices of the package bee industry. Typically package bees are shaken off of frames and pass through a queen excluder that restricts the movement of queens and drone honey bees, which are too large to pass through the openings (Laidlaw 1992). Producers who use a queen excluder should have few drones in packages, whereas those who forego the queen excluder will likely have variable amounts of drones from package to package, reflecting the variation in drone levels from the source colonies. In the current study, it seems likely that producers C and D used queen excluders, whereas the other producers did not. This resulted in four lines with low levels of drones in all packages in their shipments and two lines with highly variable drone numbers within the packages they shipped. It may be that shipping some drones in packages is not detrimental to the establishment and ultimate success of the young colony, but the extent to which colony development is delayed is unclear. The high level of drones (>20%) in one package from line 2 resulted in a 1090-g (2.4-pound) package of workers (after the weight of drones is removed).

That some *V. destructor* are shipped in packages is not surprising given the prevalence of the pest in beekeeping operations; however, the variability of MPB ratios among and within operations was noteworthy. When *V. destructor* first occurred in the United States, it was typical for producers to ship packages with a miticide impregnated plastic strip in the package cage. Once the mite became widely established, producers gave up that practice, presumably due to the added expense of the miticide application. However, the MPB ratios we observed in package shipments (when computed as standardized ether rolls) would exceed the spring treatment threshold in some regions (e.g., more than three mites per SER in Washington state; Strange and Sheppard 2001) in at least 50% of packages in five of the six lines evaluated. In this case, the expense of mite control has simply been shifted to the beekeeper. In addition, the high variance in MPB ratios within a producer (for example producers A or B) means that some packages will require treatment, whereas others from the same producer will not, and the beekeeper would be wise to sample each package to make informed treatment decisions or risk unnecessarily treating packages that are below the recommended treatment threshold.

The observed variability in varroa MPB ratios, like the variability in drone numbers, points to different production practices among package producers. The low MPB ratios observed in line 3 suggest that the producer treated production colonies with miticide shortly before making the packages, resulting in low MPB ratios. In the past, the higher MPB ratio might have been interpreted as failure to treat with miticides before making up packages. However; it is as likely that each of the other producers had different management strategies. A fall miticide application would explain intermediate or even high MPB when packages were assembled in the spring, and some producers may choose to eliminate treatments to reduce production costs. Likewise, some honey bee breeders are now selecting for mite resistant bees by foregoing chemical treatments and breeding from resistant stock (Harbo and Hoopingarner, 1997, Rinderer et al. 2000, Spivak and Reuter 2001), using integrated pest management (IPM) techniques (Pettis and Shimanuki 1999; Calderone 2000, 2005; Sheppard et al. 2003), or both, to control mite levels in the colonies they use for package production. Producer D specified in advertisements that no chemical treatments were applied for mite or disease control and instead IPM strategies were used. Both reduction in miticide use and implementation of IPM strategies would result in intermediate MPB ratio because mite levels are otherwise being managed to remain below an economic injury level.

A final and unfortunate explanation for intermediate or high MPB ratios is that the production colonies used to assemble packages may contain acaricide-resistant mites. Populations of *V. destructor* have evolved resistance to coumaphos and fluvalinate (Baxter et al. 1998, Elzen et al. 1998, Elzen et al. 1999), the active ingredients in the two most widely used acaracides in the United States. Acaricide resistance would explain the presence of *V. destructor* if the producer were relying on chemical mite control before shipping. If resistance is the explanation for the observed variation in MPB, then the mass shipments of honey bees is actually spreading resistance to acaricides. Assessing the levels of resistance in mites in packages would be valuable, but it would necessitate an alternative method of collecting mites from shipped packages that would result in live samples for bioassays.

Although the package bee industry is effective in transporting honey bees, the prevalence of *Nosema* infection in packages may indicate that packaged bees are also an effective source of pathogens. Given the levels of *Nosema* and varroa mites in the packages, it seems likely that other pests and pathogens may be present as well (Wehling 2000). Because current bee colony losses prompt fears of pathogen spread (Cox-Foster et al. 2007), it seems increasingly prudent for beekeepers to carefully evaluate shipments of bees they receive.

It should be noted that packages shipped from producer D were delayed in the mail and arrived dead in Ithaca, NY. Producer D was notified and immediately shipped a new set of packages which were apparently lost by the U.S. Postal Service. A third shipment by that producer arrived in Ithaca, NY, 2 wk later in good health, and it was included in the study. The producer paid the cost of the latter two shipments and replaced the bees at no extra cost. Two other producers (producers E and F) took our orders for packages, but they never shipped the packages either due to bee shortages or misplaced orders. We were not notified by these producers that shipments would not be forthcoming and only learned of it after calling them when the expected shipments did not arrive.

The quality of honey bee packages is largely dependent upon the colonies from which they are assembled and that depends on the management and production practices of the producer. Variable MPB ratios among producers and within some lines underscore the variability in quality beekeepers can expect when ordering package bees. Large numbers of drones in package bees highlight the willingness of some producers to sell a substandard product. Large numbers of drones serve as biological filler that adds weight and volume to the product, but they may retard the growth of the colony once installed into hives. Although it is not clear that the levels of drones in the packages we received were detrimental to colony performance, the substitution of drones for workers in honey bee packages is not a generally accepted practice. Because of results and the lack of any regulation of this industry or certification of its products, beekeepers should monitor package quality and communicate concerns with vendors to improve industry standards.

Purchasing packaged bees is a buyer beware endeavor. Although the producers included in the current study represent only four of the dozens of possible producers of packages in the United States, the packages we received illustrate the high variability that beekeepers can expect to encounter in the marketplace. Our results indicate that beekeepers should monitor the quality of incoming shipments of bees for pest and disease presence.

Acknowledgments

We thank Gil Menda and Shiri Tabenkin for technical assistance. We thank Michael Burgett and Theresa Pitts-Singer for reviewing the manuscript and providing valuable suggestions for improvement. Funding was provided, in part, by grants from the New York State Department of Agriculture and Markets and from USDA–CSREES Initiative for Future Agriculture and Food Systems (IFAFS 2001-52103-11417) (to N.W.C.).

References Cited

Baxter, J., F. Eischen, J. Pettis, W. T. Wilson, and H. Shimanuki. 1998. Detection of fluvalinate-resistant *Varroa* mites in U.S. honey bees. Am. Bee J. 138: 291.

Baxter, J. R., P. J. Elzen, D. Westervelt, D. Causey, C. Randall, F. A. Eischen, and W. T. Wilson. 1999. Control of the small hive beetle, *Aethina tumida* in package bees. Am. Bee J. 139: 792–793.

Calderone, N. W. 2000. Effective fall treatment of *Varroa jacobsoni* (Acari: Varroidae) with a new formulation of formic acid in colonies of *Apis mellifera* (Hymenoptera: Apidae) in the northeastern United States. J. Econ. Entomol. 93: 1065–1075.

Calderone, N. W. 2005. Evaluation of drone brood removal for management of *Varroa destructor* (Acari: Varroidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae) in the northeastern United States. J. Econ. Entomol. 98: 645–650.

Calderone, N. W., and H. Shimanuki. 1992. Evaluation of sampling methods for determining infestation rates of the tracheal mite (*Acarapis woodi* R) in colonies of the honey bee (*Apis mellifera*)–spatial, temporal, and spatiotemporal effects. Exp. Appl. Acarol. 15: 285–298.

Calderone, N. W., and H. Shimanuki. 1993. Distribution of the tracheal mite, *Acarapis woodi*, among the mesothoracic tracheal trunks of the honey-bee, *Apis mellifera.* Exp. Appl. Acarol. 17: 663–672.

Calderone, N. W., and R. M. Turcotte. 1998. Development of sampling methods for estimating levels of *Varroa jacobsoni* (Acari: Varroidae) infestation in colonies of *Apis mellifera* (Hymenoptera: Apidae). J. Econ. Entomol. 91: 851–863.

Cox-Foster, D. L., S. Conlan, E. C. Holmes, G. Palacios, J. D. Evans, N. A. Moran, P. L. Quan, T. Briese, M. Hornig, D. M. Geiser, et al. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science (Wash., D.C.) 318: 283–287.

De Jong, D., P. H. De Jong, and L. S. Goncalves. 1982a. Weight loss and other damage to developing worker honeybees from infestation with *Varroa jacobsoni.* J. Apic. Res. 21: 165–167.

De Jong, D., D. De Andrea Roma, and L. S. Goncalves. 1982b. A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honeybees. Apidologie 13: 297–306.

Delaplane, K. S. 1994. Installing package bees. Am. Bee J. 134: 249–251.

Delaplane, K. S., and W. M. Hood. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the southeastern USA. J. Apic. Res. 36: 125–132.

Elzen, P. J., J. B. Baxter, F. A. Eischen, and W. T. Wilson. 1999. Pesticide resistance in *Varroa* mites: theory and practice. Am. Bee J. 139: 195–196.

Elzen, P. J., F. A. Eischen, J. B. Baxter, J. Pettis, G. W. Elzen, and W. T. Wilson. 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. Am. Bee J. 138: 674–676.

Harbo, J. R., and R. A. Hoopingarner. 1997. Honey bees (Hymenoptera: Apidae) in the United States that express resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). J. Econ. Entomol. 90: 893–898.

Harrison, B. 2005. US beekeepers make history with first Australian bee imports. Am. Bee J. 145: 287–289.

Hayes, G. W. 2006. Apiary Inspectors of America question allowing Australian package bees into California. Am. Bee J. 146: 474–475.

Klee, J., A. M. Besana, E. Genersch, S. Gisder, A. Nanetti, D. Q. Tam, T. X. Chinh, F. Puerta, J. M. Ruz, P. Kryger, et al. 2007. Widespread dispersal of the microsporidian *Nosema ceranae,* an emergent pathogen of the western honey bee, *Apis mellifera.* J. Invertebr. Pathol. 96: 1–10.

Laidlaw, H. H. 1992. Production of queen and package bees, pp 989–1042. *In* J. M. Graham [ed.], The hive and the honey bee. Dadant & Sons, Hamilton, IL.

Liu, T. P., and M. E. Nasr. 1993. Preventive treatment of tracheal mites, *Acarapis woodi* (Rennie) with vegetable oil extender patties in the honey-bee, *Apis mellifera* L. colonies. Am. Bee J. 133: 873–875.

Morse, R. A., and Calderone, N. W. 2000. The value of honey bees as pollinators of US crops in 2000. Bee Culture 128: 2–15.

Pettis, J. S., and H. Shimanuki. 1999. A hive modification to reduce *Varroa* populations. Am. Bee J. 139: 471–473.

Rinderer, T. E., L. I. de Guzman, J. Harris, V. Kuznetsov, G. T. Delatte, J. A. Stelzer, and L. Beaman. 2000. The release of ARS Russian honey bees. Am. Bee J. 140: 305–307.

Sheppard, W. S., M. Gardner, S. Hasher, B. Kahkonen, M. D. Meixner, and J. P. Strange. 2003. Use of sucrose octanoate esters to control the parasitic honey bee mite *Varroa destructor.* Am. Bee J. 143: 982–985.

Shimanuki, H., and D. A. Knox. 2000. Diagnosis of honey bee diseases. USDA–ARS Handbook No. 690. Washington, DC.

Spivak, M., and G. S. Reuter. 2001. *Varroa destructor* infestation in untreated honey bee (Hymenoptera: Apidae) colonies selected for hygienic behavior. J. Econ. Entomol. 94: 326–331.

SPSS Inc. 2006. SPSS 15.0 for Windows brief guide. SPSS Inc., Chicago, IL.

Strange, J. P., and W. S. Sheppard. 2001. Optimum timing of miticide applications for control of *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) in Washington state, USA. J. Econ. Entomol. 94: 1324–1331.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. biotechniques 10: 506–513.

Wehling, W. F. 2000. Pest risk assessment: importation of adult queens, package bees and germplasm of honey bees, *Apis mellifera* L., from Australia: qualitative, pathway-initiated pest risk assessment. *In* April 2000. Plant Protection and Quarantine, Animal and Plant Health Inspection Service. U.S. Dep. of Agriculture, Riverdale, MD.

Received 3 December 2007; accepted 7 February 2008.